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**STUDY ON ANTIBACTERIAL EFFECT OF MEDLAR AND HAWTHORN COMPOUND
EXTRACT IN VITRO**

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Abstract

This paper is to study the antibacterial effect of medlar and hawthorn compound extract in vitro. Water extract method and ethanol extraction method are adopted to prepare the compound extracts, and disc diffusion method and improved test tube doubling dilution method are adopted to make the antibacterial test to the two common pathogenic bacteria in vitro, Staphylococcus aureus and Klebsiella pneumoniae. The results show that medlar and hawthorn compound extract is moderately sensitive to Staphylococcus aureus, while its inhibiting effect on Klebsiella pneumoniae is particularly significant, and the antibacterial effect of ethanol extract is better than water extract. Conclusion is that the medlar and hawthorn compound has good antibacterial effect on the two pathogenic bacteria.

Keywords: Medlar and hawthorn compound, content measuring, Antibacterial activity, Killing curve

Introduction

Medlar can nourish liver and kidney and regulate blood and dryness, and has the effects of liver protection, blood pressure decreasing, cholesterol decreasing and cosmetology. Hawthorn has the effects of appetite and digestion promoting, blood activating and stasis dissolving, anti-bacteria and inflammation diminishing, as well as body immunity improving, anti-aging, and blood sugar and blood lipid regulating. The compound composed of the two has the effects of liver and kidney nourishing, blood enriching, intellect improving and cardiovascular and cerebrovascular disease curing, as well as antibacterial and analgesic efficacy (Leung et al., 2001; Toyoda-Ono et al., 2004; Kim et al., 2000). To study its antibacterial effect, we take advantage of its rich natural resources and expand the scope of application, and this paper studies the antibacterial effect of the medlar and hawthorn compound extract in vitro.

Materials and Methods

Drugs

Medlar and hawthorn are bought from Nepstar Drugstore in Yinchuan City, Ningxia Province. Doctor Nan Yi in this college identify that they are dry and ripe fruit of Lycium barbarum L.and dry and ripe fruit of C.Pinnatifida Bge., and the specimens are stored in the Specimen Room in the Pharmaceutical College.

Experimental bacteria

S.aureus, ATCC25923 and K. Pneumonia, ATCC700603 are provided by the National Institute for the

Instruments, culture medium and reagents

Liquid chromatograph P3000 (Beijing Chuangxin Tongheng Science and Technology Co., Ltd); ZHJH-CH09B clean bench (Shanghai Zhicheng Analytical Instrument Manufacturing Co., Ltd); DHG-101-3A heating and drying oven (Gongyi Yingyu Yuhua Co., Ltd); Difunctional vapour-bathing constant temperature vibrator (Jintan Jieruier Electric Co., Ltd); M302078 bacteria turbidimeter (Beijing Zhongxi Yuanda Science & Technology Co., Ltd); Gallic acid control (110831-200803) (provided by Ningxia Institute for Drug Control)

Experimental methods

Preparation of medlar and hawthorn compound extract

Water extract: weigh 10g hawthorn and 5g medlar, respectively crush and pass through 40 mesh screen, add 10 times of distilled water, extract by heating and backflow three times, 1h each time, combine the extract solution, cool down, filter, concentrate into extractum, weigh as 9.7g, and the extractum rate is 64.7%. 50% ethanol extract: weigh 10g hawthorn and 5g medlar, respectively crush and pass through 40 mesh screen, add 10 times of 50% ethanol, extract by heating and backflow three times, 1h each time, combine the extract solution, cool down, filter, concentrate into extractum, weigh as 8.2g, and the extractum rate is 54.7%.

Chromatographic conditions

50% ethanol extract is prepared at the concentration of 1mg/ml, standard substance is gallic acid, and chromatographic conditions are: Dima C18 column (150nm * 4.6mm, 5μm), mobile phase is methanol-0.5% phosphoric acid (5:95), detection wavelength is 276nm, column temperature is 30°C, the sampling volume is 5μl, and the flow rate is 0.8 mL/min.

Drug sensitive test (Tao et al., 2009)

Take 0.2mL test bacterial solution activated and diluted to 1/2 Mcfar turbidity to evenly coat and inoculate in sterile nutrient broth agar, paste the drug disks on the inoculated agar plate, six disks for each with same distance, and each test for 3 times of parallel tests. They are inverted in culture incubator at 37°C and take out after 24h, read the diameter of each inhibition zone with vernier caliper, 3 times for each inhibition zone, and take the mean. All the parallel test data for all the inhibition zones are processed with SPSS16.0 software, and the data for each group are expressed with ($\bar{X} \pm SD$).

Minimal inhibitory concentration (MIC) determination (Mo et al., 2009)

Adopt improved test tube doubling dilution method. Water extract and 50% ethanol extract and used respectively to make two groups of parallel tests. The bacterial solution diluted to 1/2 Mcfar turbidity is diluted with nutrient broth by 1:1000, and the diluted bacterial solution is obtained. Add 1.8mL diluted bacterial solution into the first tube and add 1.0mL into other tubes, then add 0.2mL drug solution (crude drug 1g/mL) into the first tube, and after mixing, take 1.0mL to add into the second tube. Doubly diluted to the 10th tube, abandon 1.0mL, and the 11th tube is for the growth control, i.e. no drug, so as to observe whether the culture medium fits for

bacterial growth. 2mL drug solution diluted with sterile nutrient broth is added into the 12th tube, to observe whether the test drug solution is contaminated. Three times of repeated tests are made to each test bacterium. The above test tubes are placed in 37°C incubator for 2h and taken out for observation, the bacterium in the 11th tube presents turbid growth, while the 12th tube has no bacterial growth and presents transparent, the minimal drug concentration for sterile growth is determined as MIC.

Minimal bactericidal concentration (MBC) determination (Lin et al., 2003)

Use 4mm inoculating loop, the culture solutions with the drug concentration over MIC are for streak inoculation in the nutrient broth agar medium, each tube for 2 plates, they are inverted in incubator at 37°C for 18h and taken out, the minimal drug concentration with no bacterial growth or less than five colonies is determined as MBC of the drug to this bacterium.

Killing curve (Okoli et al., 2005)

Select 1/2MIC, MIC, 2MIC, 4MIC, 8MIC and 16MIC drug concentration in the test tubes, add bacterial solution and make its concentration at 105CFU/ml in the tubes, at 0, 2, 4, 6, 8, 12, 18 and 24h, fully mix the tubes, sample, dilute, count the viable bacteria with plate method, and draw the bacterial count – time curve.

Results and Discussion

Result of measuring Gallic acid content in the different extracts by HPLC

HPLC is adopted to make chromatographic analysis on Gallic acid in water and 50 ethanol extracts, there is Gallic acid in these two extracts, and its content is measured, see Figure 1.

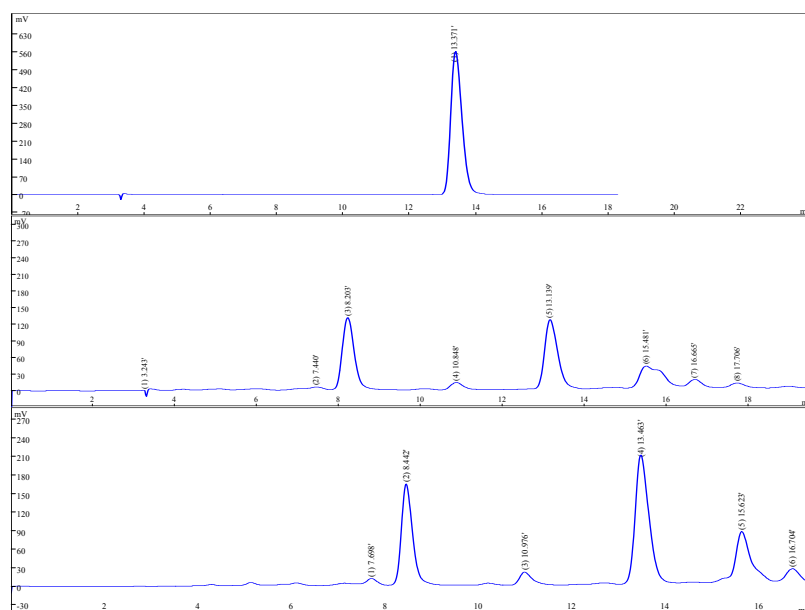


Figure 1: (a) the HPLC chromatograms of gallic acid, (b) the HPLC chromatograms of water extracts, (c) the HPLC chromatograms of 50% ethanol extracts

It can be seen from the result that Gallic acid content in water and 50% ethanol extracts is different, respectively 2.94% and 4.61%. Gallic acid content in 50% ethanol extract is significantly higher than that in water extract. Meanwhile, we find that the antibacterial activity of 50% ethanol extract is also higher than that of water extract.

Drug sensitivity measuring result

According to the determination standard for the qualitative result of anti-bacteria in vitro, the medlar and hawthorn compound extract is highly sensitive to *Staphylococcus aureus* and *Klebsiella pneumoniae*, and the antibacterial force to *Klebsiella pneumoniae* is the strongest. The results of the drug sensitive test of medlar and hawthorn compound to the two pathogenic bacteria are shown in Table 1.

Table 1: Diameter of inhibiting bacteria circle of extracts (mm)

Sample name	Diameter of inhibiting bacteria circle	
	<i>S.aureus</i>	<i>K. Pneumonia</i>
water extracts	15.28±0.13	17.21±0.24
50% ethanol extracts	19.33±1.12	23.87±0.18

MIC and MBC values of the different extracts

MIC and MBC values of the different extracts of medlar and hawthorn compound to *Staphylococcus aureus* and *Klebsiella pneumoniae* are low, which is in accordance with its inhibition zone result. MIC and MBC values of the 50% ethanol extract of medlar and hawthorn compound to the two pathogenic bacteria are lower than its water extract, as shown in Table 2.

Table 2: MIC and MBC of inhibiting bacteria of extracts (mg/ml)

Sample name	water extracts		50% ethanol extracts	
	MIC	MBC	MIC	MBC
<i>S.aureus</i>	5	100	2.5	50
<i>K. Pneumonia</i>	2.5	50	0.625	2.5

Killing curve of 50% ethanol extract

According to MIC test result, the stock solution of 50% ethanol extract is diluted respectively to six concentrations, and the killing curves for the two bacteria are drawn by taking different time points as the horizontal axis and taking the logarithms of the culture colony counts at the different time points as the vertical axis, as shown in Figure 2 and Figure 3.

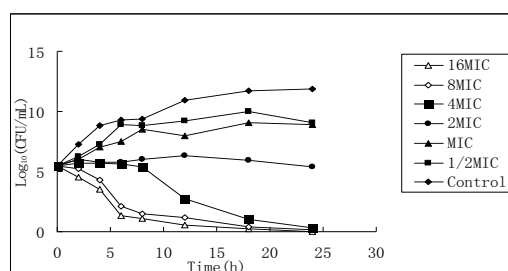


Figure 2: Killing *S.aureus* curve of 50% ethanol extracts

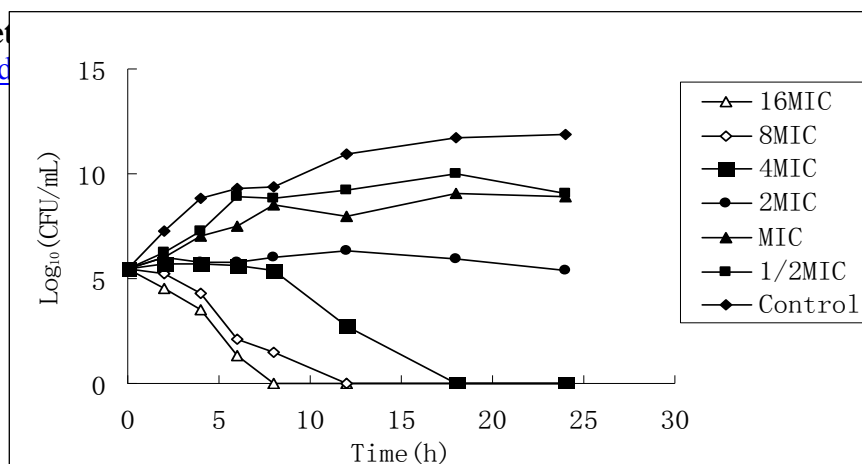


Figure 3: Killing K. Pneumonia curve of 50% ethanol extract

It can be seen from FIG2 that for the effect on *Staphylococcus aureus*, as the extract concentration increases, bacterial growth at 24h is completely inhibited, but it cannot completely kill the bacteria; it can be seen from FIG3 that the time for killing *Klebsiella pneumoniae* turns shorter, the killing effect increases and presents concentration dependence, and when the concentration increases above MIC value, it can completely kill the bacteria.

Hawthorn, mainly produced in Northern China, is a commonly used traditional Chinese medicine. There are more than 150 substances found and separated from hawthorn at present, and the main components are mostly phenolic compounds, such as apigenin, luteolin, quercetin, (+)-catechin and (-)- epicatechin, or dimer and polymer forms, such as procyanidin B2, B4 and B5, ursolic acid, and corosolic acid; cycloartane form, such as cycloartenol, oleanolic acid and crataegolic acid; also including a large quantity of organic acids, such as benzoic acid, Gallic acid, protocatechuic acid, chlorogenic acid, β -coumaric acid, caffeic acid, ferulic acid, anisic acid, vanillic acid, syringic acid, gentisic acid, etc (Melikouglu et al., 1999; Melikoglu et al., 2000; Mousallamy et al., 1998; Ulla et al., 2002; Woo et al., 1994; Carmen et al., 2001; Sun et al., 2000; Garcia et al., 1997). Hawthorn phenolic compound has many pharmacological activities, such as lowering blood lipid, lowering blood pressure, helping digestion, anti-oxidation, anti-bacteria, anti-tumor, etc (Essaady et al., 1996; Choi et al., 2000; Hsu et al., 1997; Lee et al., 1994). Medlar mainly contains betaine, LBP, scopoletin, free amino acids, vitamins, etc, and it has sweet taste and moderate nature. Medlar has the effects of immune function strengthening, anti-bacteria, anti-aging, liver protection, blood sugar lowering, anti-tumor, blood pressure lowering and anti-oxidation (Kim et al., 1997; Peng et al., 2001; Leung et al., 2001; Toyoda-Ono et al., 2004; Kim et al., 2000).

Experimental results show that medlar and hawthorn compound extract has strong inhibitory effect on *Staphylococcus aureus* and *Klebsiella pneumoniae*. In general, the ethanol extract of medlar and hawthorn compound has better antibacterial effect than the water extract, and in coincidence, the main component gallic acid content in the ethanol extract is significantly higher than that in the water extract, and the reason may be related to that the extraction with ethanol as the solvent and coordinated with hot reflux can greatly increase the extraction rate of phenols and other active components in the drugs, so proper processing technology can be selected according to the difference in the nature of the active components in traditional Chinese medicine, to make it maximize the efficacy (Liu et al., 2009).

This experiment makes some basic studies on the antibacterial effect of medlar and hawthorn compound and makes HPLC to the main components of the extract, but its mechanism of antibacterial effect needs more in-depth study in the future. In recent years, along variety increase and wide application of antibiotics, the problem of bacterial resistance is growing, and adverse drug reaction is increasing. As chemosynthetic drugs are hard to develop and have significant toxic and side effects, while Chinese medicines and Chinese herbal compounds have less resistance to the majority of bacteria (Yu et al., 2007). Therefore, the research and development of

antibacterial Chinese medicines has important significance on solving the problems of resistant strain production and antibiotics abuse (Wang et al., 2007).

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